

User Manual

NT.Robotic Autosampler System

for Prometheus NT.Plex

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Preface

This manual is a guide for using the NT.Robotic Autosampler (NT.RA) System integrated with a Prometheus NT.Plex. It instructs first-time users on how to use the NT.RA system, and serves as a reference for experienced users. For detailed information on how to use the Prometheus NT.Plex, please refer to the corresponding user manual.

Before initializing the NT.RA system, please read this instruction manual carefully, and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. When not using the instrument, keep this manual in a safe place. If this manual becomes lost, order a replacement from NanoTemper Technologies GmbH.

Notices

1. NanoTemper Technologies shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of product use.
2. Prohibitions on the use of NanoTemper Technologies software:
 - Copying software for other than backup
 - Transfer or licensing of the right to use software to a third party
 - Disclosure of confidential information regarding software
 - Modification of software
 - Use of software on multiple workstations, network terminals, or by other methods
3. The contents of this manual are subject to change without notice for product improvement.
4. This manual is considered complete and accurate at publication.
5. This manual does not guarantee the validity of any patent rights or other rights.
6. If a NanoTemper Technologies software program has failed causing an error or improper operation, this may be caused by a conflict from another program operating on the laptop (PC). In this case, take corrective action by uninstalling the conflicting product(s).
7. NanoTemper is a registered trademark of NanoTemper Technologies GmbH in Germany and other countries.

1. Safety Considerations

1.1. General Information and Safety Symbols

To ensure operation safety, this instrument must be operated correctly. Carefully read the following explanations to fully understand all safety precautions in this manual before operating the instrument. Please take a moment to understand what the signal words **WARNING!**, **CAUTION** and **NOTE** mean in this manual.

WARNING!

A **WARNING!** indicates a potentially hazardous situation which, if not avoided, may result in serious injury or even death.

CAUTION

A **CAUTION** indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. **CAUTION** may also be used to alert against damaging the equipment or the instrument.

Do not proceed beyond a **WARNING!** or **CAUTION** notice until you understand the hazardous conditions and have taken appropriate steps.

NOTE

A **NOTE** provides additional information to help the operator achieve optimal instrument and assay performance.



Read user manual label. This label indicates that you have to read the manual before using the system. This label is positioned at the back of the device.



Warning symbol. This symbol indicates that it is mandatory to wear safety goggles when operating the system. The label is positioned on the door of the enclosure.



Warning symbol. This symbol indicates a surface that can heat up and cause burn injuries. This warning label is positioned on the sample tray.



Warning symbol. This symbol indicates moving parts that pose a risk of injuring hands and fingers. This warning label is positioned on the robotic arm.

It is important to choose a suitable location in the laboratory that provides sufficient space and meets all requirements. Please refer to the instrument dimensions and the requirements listed below.

The Prometheus NT.Plex with NT.Robotic Autosampler must not be exposed to unusual temperature variations, such as by direct sunlight, near a window, heating or air-conditioning duct. Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment and machine tools. Avoid strong magnetic fields and sources of high frequency as well as dust and corrosive gas.

Choose an area of floor that is suitably level and that can carry the total weight of the system.

1.2. Safety Instructions

Always use the NT.RA system according to the instructions in this user manual. If the system is used in ways not specified in this user manual, the safety precautions provided by NanoTemper Technologies may not be effective as intended.

Always use the correct consumables (see 5.2.4) to ensure that the system works as intended.

1.2.1. General

WARNING! Operate the system and all its components only with the provided external power supplies. Only use the provided cables and plugs. Failure to comply may result in a risk of electric shock and fire.

CAUTION The system may only be run while the wheels are locked.

CAUTION The system has to be installed in a way that does not hinder access to the external power supplies and their power plugs.

CAUTION Connect the system power supplies in a way that avoids tripping hazards.

CAUTION Do not use additional extension cords. Damaged cords, plugs or cables need to be replaced immediately. Failure to comply may result in a risk of personal injury or damage to the instrument.

CAUTION Only NanoTemper Technologies staff may service the system and all its components. Turn off the power switches and unplug the power cords before servicing the instrument, unless instructed otherwise.

CAUTION Mechanical moving parts within the system can pinch or injure your hands or fingers. Do not touch or open the instrument while parts are moving.

CAUTION Use only certified cables and plugs.

1.2.2. Prometheus NT.Plex

WARNING! Operate the Prometheus Series instrument only with the provided external power supply. Only use the provided cables and plugs. Failure to comply may result in a risk of electric shock and fire.

CAUTION The Prometheus Series instrument has to be installed in a way that does not hinder access to the external power supply and its power plug.

CAUTION Connect the Prometheus Series instrument power supply in a way that avoids tripping hazards.

CAUTION Do not use extension cords. Damaged cords, plugs or cables need to be replaced immediately. Failure to comply may result in a risk of personal injury or damage to the instrument.

WARNING! Do not operate the Prometheus Series instrument with substances or under conditions that pose a risk of explosion, implosion or release of gases. Do not use the instrument with hazardous or infectious substances.

CAUTION Use only aqueous samples for analysis in the instrument.

CAUTION The Prometheus Series instrument weighs approx. 30 kg. Two people are required for transport. Moving the instrument alone entails a risk of personal injury or damage to the instrument.

CAUTION Do not open the instrument manually or anywhere other than the sample loading drawer. Opening entails a risk of personal injury or damage to the instrument and may only be done by NanoTemper Technologies staff.

CAUTION Only NanoTemper Technologies staff may service the instrument. Turn off the power switch and unplug the power cord before servicing the instrument, unless instructed otherwise.

CAUTION The instrument contains a UV-LED. The UV-LED emits invisible ultraviolet radiation (UVB radiation) when in operation, which may be harmful to eyes and skin, even at brief periods of exposure. Do not look directly into the UV-LED during operation. If used as intended, the instrument emits no UV radiation.

CAUTION Mechanical moving parts within the instrument can pinch or injure your hands or fingers. Do not touch or open the instrument while parts are moving.

CAUTION The display pane is made of glass. Broken glass can injure your hands or fingers.

CAUTION The sharp edges of the Prometheus NT.Plex chipholder thermal element pose a risk of injury to hands and fingers.

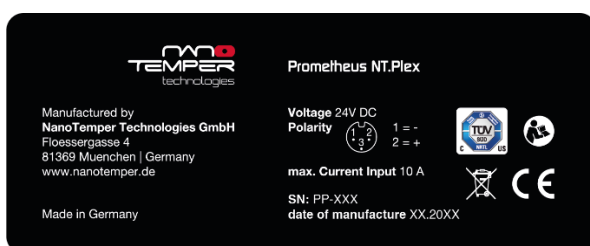
CAUTION The instrument contains a temperature regulator to control the sample temperature. Some accessible parts of the instrument can reach temperatures of up to 60 °C. Don't touch the temperature-controlled parts of the instrument when the temperature controller is set to high temperatures.

CAUTION Do not use the instrument at ambient temperatures below 15 °C.

CAUTION Use the instrument only at noncondensing conditions (0–80 % humidity, 15-30 °C). At very high humidity levels, even normal operating temperatures may result in condensation and corrosion.

CAUTION Turn off the instrument when not in use.

CAUTION Do not use ethanol or other types of organic solvents to clean the instrument as they may remove the instrument paint.



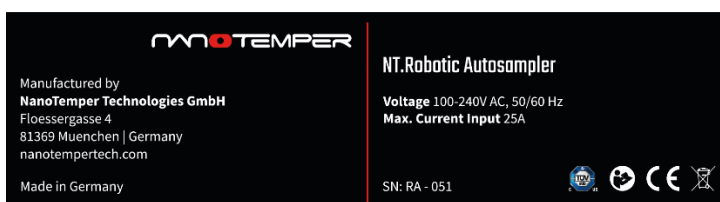
Identification label. This label is positioned at the rear panel of the device.

1.2.3. NT.Robotic Autosampler

WARNING! Glass capillaries can burst if handled improperly by the robotic arm, or in the event of a collision and subsequent closing of the robotic arm gripper. Glass splinters can injure eyes and skin. Wear appropriate safety goggles when operating the NT.Robotic Autosampler.

WARNING! The robotic arm has a release button located on the underside of the shoulder joint. Pushing this button will cause the robotic arm to release all joints and drop down. The weight of the robotic arm can injure hands and fingers and damage NT.RA components. Do not push this button unless necessary. If you do push this button, be careful to catch the robotic arm to prevent it from falling. Be careful not to injure your hand or fingers.

CAUTION Doors and door handles of the enclosure can pinch fingers. Handle carefully.



Identification label. This label is positioned at the rear panel of the device.

2. Regulatory Statement

The NT.Robotic Autosampler as a complete system is certified according to IEC 61010-2-081 (Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes).

The individual components of the system, for example the Prometheus NT.Plex or the robotic arm, are certified according to additional standards and regulations. For more information, please refer to the respective component's user manual.

3. System Specifications

3.1. Dimensions

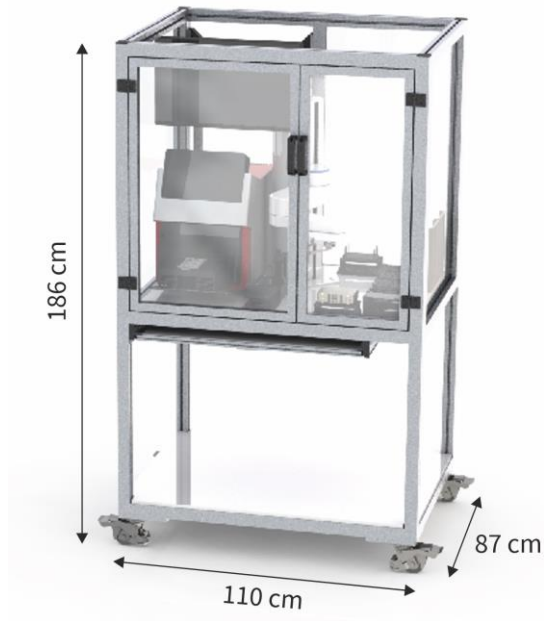


Figure 1: The dimensions of the NT.RA system.

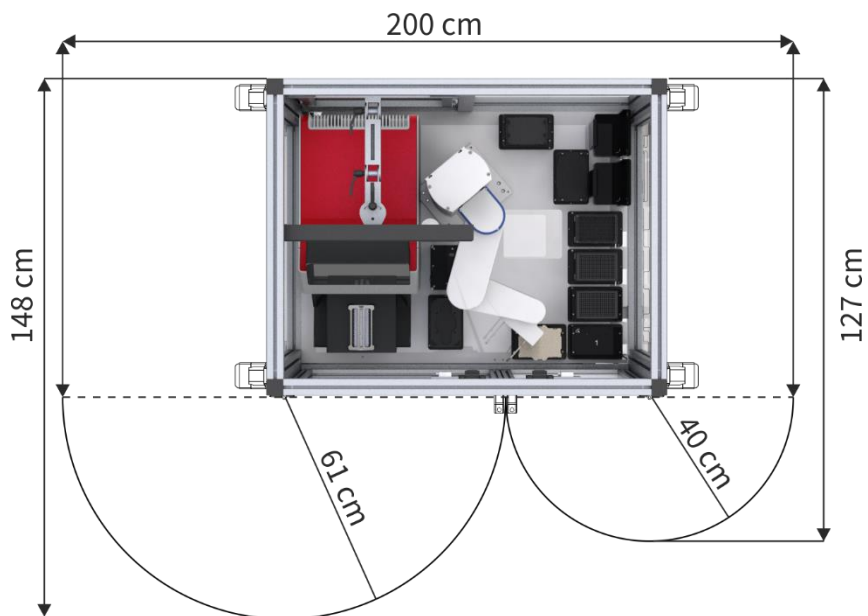


Figure 2: The dimensions of the NT.RA enclosure with doors open (top-down view). Ensure that there is sufficient space for the doors to be opened fully to both sides, and to access the wheels to put the brakes on.

3.2. Technical specifications and operating conditions

Electrical specifications			
Input voltage	100 VAC (-10%) to 240 VAC (+10%) 90 VAC to 264 VAC		
Input frequency	50 / 60 Hz \pm 5%		
Electrical input current	25 A		
Overvoltage category	2		
	Output power supply	Maximum power consumption	Inrush current (230VAC / cool start)
Prometheus NT.Plex	24 VDC, 10.41 A max	300 VA	120 A
NT.Robotic Autosampler	-	200 VA	100 A
NT.Tilting Unit	24 VDC, 3.0 A max	25 VA	20 A
Control PC	-	150 VA	50 A
Plate Temperature Control (optional)	-	650 VA	100 A
Other specifications			
Weight	225 kg max		
Pollution degree	2		
Environmental conditions			
Operating temperature range	15°C to 30°C (indoor use only)		
Relative humidity	0% to 80%, noncondensing		
Operating altitude	max 2000 m		
Noise	< 65dB(A)		

The system should be located within easy reach of standard electrical outlets. We recommend the use of switchable power strips (not included). The standard configuration requires 5 sockets; an additional socket is required if the configuration contains the optional Plate Temperature Control.

It is recommended to connect the system components to two separate power circuits. Depending on the type of power outlets, breakers and fuses, inrush currents and maximum currents might overload a single circuit. We therefore recommend connecting the robotic arm itself to a separate power circuit from the other components.

The line disconnecter needs to be accessible at any time.

Please provide a suitable trash can (maximum height 50 cm).

4. Limited Warranty

Products sold by NanoTemper Technologies, unless otherwise specified, are warranted to be free of defects in materials and workmanship for a period of one year from the date of installation. If any defects in the product are found during this warranty period, NanoTemper Technologies will repair or replace the defective part(s) or product free of charge.

THIS WARRANTY DOES NOT APPLY TO DEFECTS RESULTING FROM THE FOLLOWING:

1. IMPROPER OR INADEQUATE INSTALLATION.
2. IMPROPER OR INADEQUATE OPERATION, MAINTENANCE, ADJUSTMENT OR CALIBRATION.
3. UNAUTHORIZED MODIFICATION OR MISUSE.
4. USE OF UNAUTHORIZED CAPILLARIES AND CAPILLARY TRAYS.
5. USE OF CONSUMABLES, DISPOSABLES AND PARTS NOT SUPPLIED BY AN AUTHORIZED NANOTEMPER DISTRIBUTOR.
6. CORROSION DUE TO THE USE OF IMPROPER SOLVENTS, SAMPLES, OR DUE TO SURROUNDING GASES.
7. ACCIDENTS BEYOND NANOTEMPER'S CONTROL, INCLUDING NATURAL DISASTERS.

This warranty does not cover consumables like capillaries, reagents, labeling kits and the like.

The warranty for all parts supplied and repairs provided under this warranty expires on the warranty expiration date of the original product. For inquiries concerning repair service, contact NanoTemper Technologies after confirming the model name and serial number of your NanoTemper Technologies instrument.

5. NT.Robotic Autosampler System (NT.RA)

5.1. General

The NT.Robotic Autosampler (NT.RA) in combination with a Prometheus NT.Plex instrument is a fully automated platform for high-throughput protein stability measurements with minimal user hands-on time. This user manual covers the NT.RA system, its use and dedicated software packages. For information on nanoDSF measurements and the Prometheus instrument series, please consult the respective user manual. For more details on the robotic arm please refer to the manual by the robotic arm manufacturer (Precise Automation PreciseFlex 400 Sample Handler).

5.1.1. Setup and Installation

The NT.RA system may only be set up, installed and taught by trained NanoTemper Technologies staff. During installation, the teachpoints (positions) listed in 5.2 are taught to the robotic arm. If you notice a misalignment that requires re-teaching, please contact your NanoTemper Technologies representative for assistance.

5.1.2. What's in the Box

NT.RA system component	Description
Table and enclosure	Provides enough space to house the complete NT.RA system and a Prometheus NT.Plex instrument
Desktop PC with screen, keyboard and mouse	The screen can be mounted within the enclosure or separately on a provided stand
Robotic arm	PreciseFlex 400 Sample Handler
Teaching jig	For robot teaching purposes
Chip container storage shelf	For storing an open capillary chip container during measurements
Chip container lid storage shelf	For storing the lid of the open capillary chip container during measurements
2 stackers	For storing enough capillary chip containers for maximum hands-off time
4 storage shelves for microtiter plates	For storing assay plates during measurements. If the system is equipped with the Plate Temperature Control Option, these storage shelves can be temperature-controlled via the provided control software.

4 microtiter plate lids	Steel lids for covering assay plates during measurements
Storage shelf for microtiter plate lids	For storing unused plate lids
Storage shelf for NT.Plex lid	For storing the NT.Plex lid during sample loading. The bottom shelf can also be used to store the teaching jig when not in use.
NT.Tilting Unit	Holds the assay plate for sample loading
Trash can	To position below cut-out in table. Garbage bags are not included but recommended.
2 power supplies and associated cables	For Prometheus NT.Plex and NT.Tilting Unit
Separate power cables	For connecting the robotic arm to power, and to the safety lock mechanism of the enclosure
2 network cables	For connecting the PC to the robotic arm, and the PC to the Prometheus NT.Plex
USB cable	For connecting the NT.Tilting Unit to the PC
Multi-outlet power strip	-
Optional: Inheco Multi TEC Control	Blue box; only included with NT.RA systems equipped with Plate Temperature Control

5.2. Configuration and Teachpoints

During installation, the teachpoints (positions) listed here are taught to the robotic arm. If you notice a misalignment that requires re-teaching, please contact your NanoTemper Technologies representative for assistance.

The NT.RA standard configuration, its components and associated teachpoints are illustrated in (top-down view).

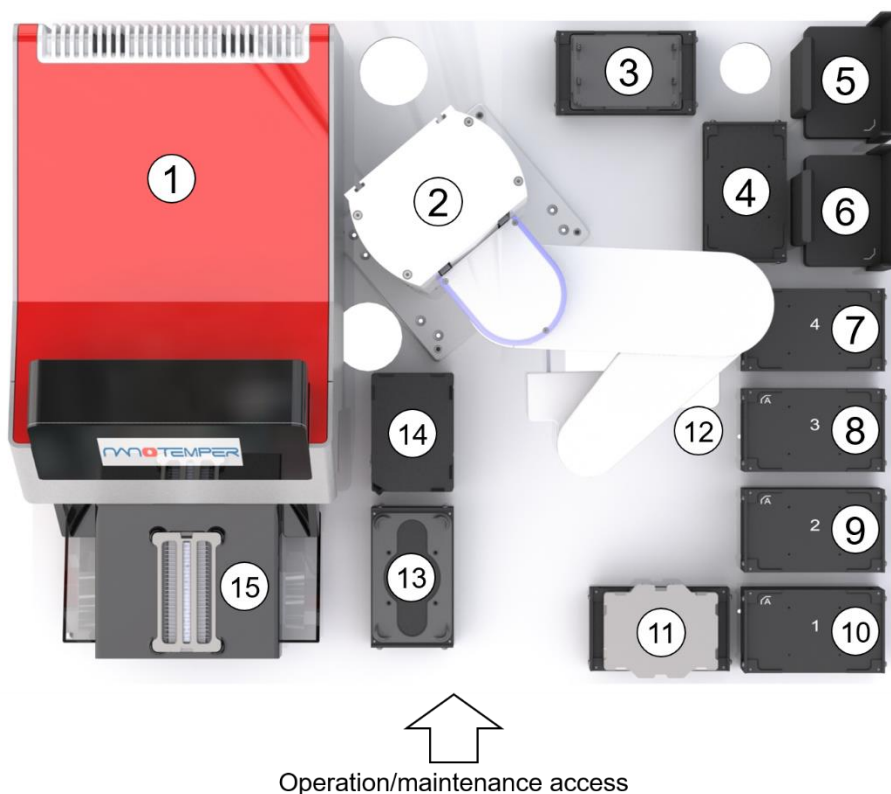


Figure 3: Top-down view of the NT.RA standard configuration, components and teachpoints.

#	NT.RA system component/position	Name of teachpoint(s)
1	Prometheus NT.Plex	see #15
2	Robotic arm	-
3	Capillary chip container lid storage	ContainerLid_Storage
4	Capillary chip container storage	Container_Storage
5	Stacker 2	Stacker_2_BottomContainer Stacker_2_TopContainer
6	Stacker 1	Stacker_1_BottomContainer Stacker_1_TopContainer
7	Storage position for microtiter plate (MTP) 4	Plate_4
8	Storage position for MTP 3	Plate_3
9	Storage position for MTP 2	Plate_2
10	Storage position for MTP 1	Plate_1
11	Storage position for MTP plate lids	PlateLid_Storage
12	Waste cut-out (trash can below table)	-
13	Storage position for NT.Plex lid	PlexLid_Storage
14	NT.Tilting Unit	Tilting_Plate Tilting_BottomRow Tilting_TopRow
15	Open sample drawer of the Prometheus NT.Plex	Plex_1_Lid Plex_1_Chip

5.2.1. Enclosure, Safety Lock and Wheels

The Prometheus NT.Plex with NT.Robotic Autosampler includes a custom-built laboratory bench with an enclosure for the NT.Robotic Autosampler and a pull-out shelf for keyboard and mouse.

The enclosure features a safety lock at the top. This ensures that the robotic arm will stop all movement immediately when the door is opened. Opening the door pauses the robotic arm's task, but it does not cancel it. Movement resumes after the door is closed again.

The enclosure is set on lockable wheels for convenient relocation of the NT.RA system. Use the Prometheus NT.Plex and the NT.RA system only while the wheels are locked. After relocation, lock the wheels and ensure that all four legs are placed firmly on the ground. Use the screws to adjust individual legs if necessary.

5.2.2. Robotic Arm

All information needed for operating the robotic arm in the context of the NT.Robotic Autosampler is given in this manual. For more details and specifications of the robotic arm itself, please refer to the manual of the manufacturer (Precise Automation PreciseFlex 400 Sample Handler).

Turn on the robotic arm by using the On/Off switch at its base. Green light at the switch indicates "on" state. The robotic arm will initialize the gripper with a close-open motion. The robotic arm can be powered off or kept on between measurements.

The robotic arm has four points for movement, also called joints (see Figure 4): the shoulder, elbow, and wrist (gripper rotation) move the gripper in x and y directions, while movement up and down the base adjusts z direction. The gripper is able to move (grasp) microtiter plates (MTPs), MTP lids, capillary chips, capillary chip containers and their lids and the NT.Plex lid.



Figure 4: The robotic arm anatomy and joints. (1) z column, (2) shoulder, (3) elbow, (4) wrist and (5) gripper and gripper fingers.

WARNING! The robotic arm has a release button located on the underside of the shoulder joint. Pushing this button will cause the robotic arm to release all joints and drop down. The weight of the robotic arm can injure hands and fingers and damage NT.RA components. Do not push this button unless necessary. If you do push this button, be careful to catch the robotic arm to prevent it from falling. Be careful not to injure your hand or fingers.

The robotic arm is controlled by the software. While the robotic arm is moving to execute orders, three events can stop it:

- Opening the door of the enclosure pauses the robotic arm's movement but does not cancel it. The robotic arm's joints **lock**. The NT.Plex continues to measure the current chip. Movement resumes after the door is closed again.
- Failure to find or grasp an object causes the arm to pause and its joints to **lock**. An error message pops up on screen with instructions. Follow the instructions and click *OK* to resume. The NT.Plex continues to measure the current chip.
- An unexpected collision causes the robotic arm to stop and go into force-free mode, meaning all of its joints are **unlocked**. The gripper closes immediately and rapidly. If the gripper is holding a capillary chip at the time, the chip and its capillaries may burst. All current and following tasks are canceled and cannot be resumed. An error message pops up on screen informing the user what happened. The NT.Plex continues to measure the current chip.

When the robotic arm stops with its joints **unlocked** (force-free mode), the user can manually move it freely into the safe zone (see Figure 5). The robotic arm will remain in any position it is manually moved to. A new experiment can be started from the software.

When the robotic arm stops with its joints **locked**, it is still able to resume its previously interrupted movement. Moving the robotic arm manually in this situation has the same consequences as a collision: the joints unlock, the gripper closes, and all tasks are canceled. Therefore, manually moving the robotic arm during a measurement is recommended/necessary only after a collision.

WARNING! Glass capillaries can burst in the event of a collision and subsequent closing of the robotic arm gripper. Glass splinters can injure eyes and skin. Wear appropriate safety goggles when operating the NT.Robotic Autosampler.



Figure 5: The robotic arm's safe zone is indicated in green. The robotic arm is safe when its wrist is in the safe zone. As a rule of thumb, manually move the robotic arm's gripper so that the gripper fingers are located above the waste.

In case of a failed self-initialization of the robotic arm, restart the NT.RA system and all its components. In case initialization still works, follow the following steps:

1. Shut down the robotic arm, the NT.Tilting Unit, and the Prometheus NT.Plex.
2. Move the robotic arm in the safe zone with the gripper above the waste.
3. Restart the PC.
4. Switch on the robotic arm, the NT.Tilting Unit, and the Prometheus NT.Plex.

5.2.3. Teaching Jig

The NT.RA system contains a teaching jig for robot teaching purposes (Figure 6). It can be stored in the bottom shelf of the NT.Plex lid storage. The teaching jig is only needed for initial setup and potential re-teaching of positions/teachpoints. If you notice a misalignment that requires re-teaching, please contact your NanoTemper Technologies representative for assistance.

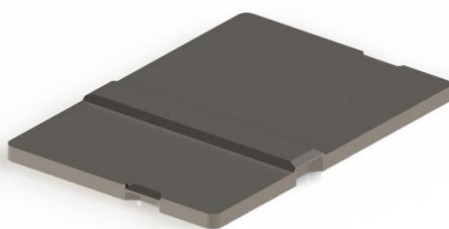


Figure 6: The NT.RA teaching jig.

5.2.4. Microtiter Plates, Lids and Their Storage Positions

The NT.RA allows for storage of up to 4 microtiter plates (MTPs) with plate lids. The NT.RA is compatible with 384 well plates manufactured by Greiner Bio-One (REF: 784201). If your setup requires the use of different plates, contact NanoTemper Technologies for information on potential solutions.

Position the MTPs on the designated plate positions labeled 1-4. The correct plate orientation is indicated. Always cover all plates with the supplied steel lids before starting the experiment. Do not use lids other than those provided with the system. Stack the remaining lids in the MTP plate lids storage position. This position is also used by the NT.RA for temporary storage of the lid of the currently used MTP during a measurement.

If necessary, clean the lids with pure water or a solution of up to 70% ethanol. Please be aware that the lids will reduce evaporation, but not eliminate it.

Plate positions exist in standard and temperature-controlled configuration. For details, see Figure 7 and section 6.3.

NOTE: Measurements will always be performed from plate 1→4 and lowest to highest rows (A→P).

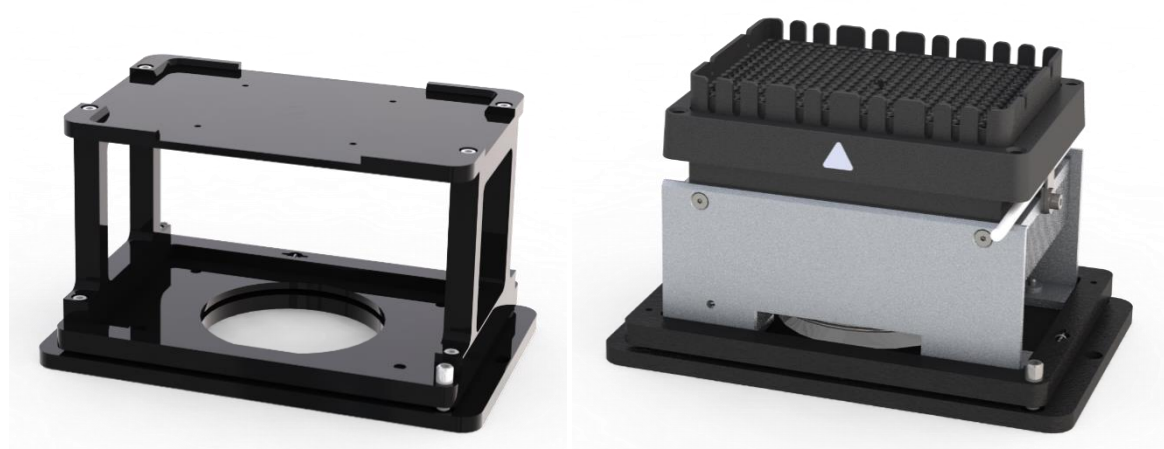


Figure 7: Plate positions in standard configuration (left) and with Plate Temperature Control (right; see section 6.3).

5.2.5. Capillary Chip Container Stackers

The two stackers hold up to 4 capillary chip container boxes each (64 capillary chips in total). Note that the topmost containers protrude above the top of the stackers.

NOTE: Before filling capillary chip containers into the stackers, remove the sealing tape and any remaining glue so the lid can be easily opened by the robotic arm.

Ensure that chip containers are fully filled with 8 chips per container before starting an experiment. When starting an experiment, the NT.RA software will automatically notify the user of the number of chip containers needed for the experiment and where to place them.

5.2.6. Capillary Chip Container Storage Position

The container storage position is used by the NT.RA for temporary storage of the currently used capillary chip container during a measurement. Ensure that the container storage position is empty before starting an experiment.

NOTE: If the chip container is not empty at the end of the measurement, it will remain in this position and needs to be removed before starting the next experiment.

5.2.7. Capillary Chip Container Lid Storage Position

The container lid storage is used by the NT.RA for temporary storage of the lid of the currently used capillary chip container during a measurement. Ensure that the container lid storage position is empty before starting an experiment.

5.2.8. NT.Plex Lid and Storage

The NT.Plex lid is used to keep loaded capillary chips firmly in place on the thermal element during a measurement. During sample loading, the NT.RA temporarily stores the NT.Plex lid on the NT.Plex lid storage position.

The NT.Plex lid is automatically stored inside of the Prometheus NT.Plex when the NT.RA system is not in use. Before starting an experiment, ensure that this is the case.

5.2.9. NT.Tilting Unit

The NT.RA positions MTPs on the NT.Tilting Unit for sample loading. The NT.Tilting Unit tilts the MTP to a vertical angle to facilitate entry of the samples into the capillaries.

5.3. Cleaning

All surfaces except for the Prometheus NT.Plex can be cleaned with water or up to 70% ethanol. Ensure that all power is turned off. Use a damp cloth to wipe surfaces.

6. Operating the NT.RA

6.1. General Considerations

NT.RA system installation and repairs should only be performed by trained NanoTemper Technologies staff. In addition to this user manual, please also consult the user manuals of the Prometheus NT.Plex and the robotic arm (PreciseFlex 400 Sample Handler) to learn about potential hazards and risks before use. For questions, please contact support@nanotempertech.com.

WARNING! Prior to initializing the NT.RA system or starting a measurement, make sure the space inside the enclosure is clear of obstructions. Collisions with foreign objects can lead to damage and misalignment of the instrument.

6.2. Sample Preparation

Prepare at least 20 μ l of sample per well in a 384 well microtiter plate (MTP). The NT.RA is compatible with 384 well plates manufactured by Greiner Bio-One (REF: 784201). If your setup requires the use of different plates, contact NanoTemper Technologies for information on potential solutions. For more details on sample properties and preparation, refer to the Prometheus series instruments user manual.

Sample plates used with the NT.RA need to be covered with the supplied steel lids. Other lids are not compatible and cannot be used with the system.

6.3. Plate Temperature Control (Optional)

The Plate Temperature Control Option enables individual temperature control of all 4 plate positions (see Figure 7) from 4 °C to 20 °C. For recommendations on plate temperatures with regards to ambient temperature and air humidity, please see the sections below.

Existing NT.RA systems (cat# NT-RA02) can be upgraded with the Plate Temperature Control Option (cat# NT-PTC). For more information, please contact your NanoTemper specialist.

6.3.1. Temperature Range

Cooling

Please ensure that your lab is condensation-free for the chosen temperature. Condensation is especially a risk at high ambient temperatures and high humidity in combination with low cooling temperatures. Condensation may impact the measurement and damage the device.

We recommend a cooling temperature of 8 °C to minimize the risk of condensation as opposed to 4 °C.

Room Temperature

For experiments where constant room temperature conditions are desired, choose a plate temperature of 20 °C.

The MTP lids supplied by NanoTemper Technologies minimize evaporation for the available temperature range.

For laboratories with low ambient temperatures: We recommend choosing the plate temperature of the Plate Temperature Control depending on the ambient temperature in your lab. Plate temperatures higher than ambient temperatures may lead to sample loss by evaporation.

6.3.2. Operation

The temperature is set via PR.AutoThermControl or PR.AutoChemControl software and can be set individually for each plate position. It can also be deactivated for each plate position individually if needed.

By default, the Plate Temperature Control is activated for all 4 plate positions upon login to the system. It will start heating/cooling immediately, using the last used temperature settings.

Cooling from room temperature to 8 °C takes approximately 10 min. It is recommended to store sample plates on the plate positions after the plate temperature has been reached.

The plate position temperatures will be monitored during the course of the entire measurement series. The temperature trace for each MTP row from start of the measurement series until loading of the corresponding capillary chip into the Prometheus NT.Plex will be visible in the corresponding experiment in PR.ThermControl software. Please refer to the PR.ThermControl manual for details.

6.4. Measurement Workflow

The general workflow of a measurement is described below. Variations may apply depending on the specific experimental setup and instrumental configuration.

The user places the sample-containing microtiter plate (MTP) in MTP storage position. The robotic arm transfers the plate onto the NT.Tilting Unit, retrieves a capillary chip from a capillary chip container, delivers the capillary chip to aspire the samples, transfers the chip onto the drawer of the Prometheus NT.Plex, and places the NT.Plex lid to hold the capillary chip in place. The drawer closes automatically and the measurement is started. The robotic arm then returns the MTP to storage and replaces the lids onto the capillary chip container and the MTP. Once the experiment is completed, the robotic arm automatically discards used chips and empty chip containers, and stores the NT.Plex lid inside the NT.Plex.

6.5. Standard Operating Procedure

1. Turn on the Prometheus NT.Plex and the NT.Tilting Unit by using the On/Off button at the left leg of the system table. Green light indicates “on” state.
2. Turn on the robotic arm by using the On/Off switch at its base. Green light indicates “on” state. The robotic arm will initialize the gripper with a close-open motion.
3. Turn on the PC by using the power button on its front.
4. Move the robotic arm into the safe zone. Remove any objects the robotic arm may be holding.
5. Start the software of your choice (PR.AutoThermControl or PR.AutoChemControl).
6. Design the experiment in the software.
7. Cover each assay plate with a supplied steel MTP lid and place the covered plates in the designated MTP storage positions.
8. Store remaining MTP lids in the MTP lid storage position.
9. Ensure that the chip container storage position, chip container lid storage position, NT.Plex lid storage position, and NT.Tilting Unit are empty.
10. Make sure that the NT.Plex lid is inside the NT.Plex.
11. Place the exact number of chip containers needed for the assay in the stackers, starting with stacker 1 (as defined by the software, see step 13). Use only completely full chip containers (8 chips inside). When starting with a new chip container, make sure to remove the sealing tape and any remainder of the glue so the lid can be easily opened by the robotic arm.
12. Close the enclosure door.
13. Start the measurement by clicking OK in the setup information window in the software. A pop-up window will display reminders of steps 7 through 12 and specify the number of capillary chip containers needed in step 11.
14. After the measurement is finished, start over or turn off all system components (see steps 1-3).

NOTE: In case a measurement is aborted via the control software before completion, the NT.RA will stop at the next target position, and the Prometheus NT.Plex will stop measuring immediately. In an event when the door to the enclosure is opened while an experiment is running, the NT.Plex measurement will continue. The NT.RA will pause immediately and resume after the door is closed again.

6.6. Manual Use of the Prometheus NT.Plex

The Prometheus NT.Plex can be used manually even when coupled to an NT.RA system. Open the enclosure as needed and use the Prometheus touch display to open/close the sample drawer. Use the regular software packages (PR.ThermControl, PR.ChemControl and PR.TimeControl) for running measurements.

6.7. Long Term Measurements and Use of the High Temperature Option in Combination with the NT.RA

Long term measurements > 3 h or thermal unfolding experiments which exceed a temperature of 95 °C require sealing of capillaries to prevent air-bubble formation and/or extensive evaporation. Since automated capillary sealing with the NT.RA is not possible, such measurements have to be performed manually. See 6.6 for manual use of Prometheus NT.Plex when coupled to an NT.RA system.

For fast and convenient capillary sealing, NanoTemper Technologies provides Capillary Sealing Paste (cat# PR-P001) and Capillary Sealing Applicators (cat# PR-P002). The sealing paste is chemically inert, silicone-free, and water-insoluble.

Measurements with durations below 3 h and temperatures up to 95 °C do not require capillary sealing at all due to the patented, sealing-free setup of Prometheus instruments.

7. NT.RA Software

The NT.RA is supported by two intuitive control software packages; **PR.AutoThermControl** software dedicated to thermal unfolding experiments, and the **PR.AutoChemControl** software dedicated to chemical unfolding experiments. For regulated environments, PR.AutoThermControl can be replaced by **PR.AutoThermControl-CFR**, which contains additional features to enable compliance with 21 CFR part 11 (FDA) and Annex 11 (EU) regulations. The following section explains features common to all packages as well as differences between them.



PR.AutoThermControl



PR.AutoChemControl

7.1. Backward compatibility

Measurements collected with all previous versions of PR.AutoThermControl, PR.AutoChemControl, and PR.AutoThermControl-CFR software are compatible with the current version.

NanoTemper Technologies provides support for all current and previous versions of these software packages.

7.2. Measurement Layouts

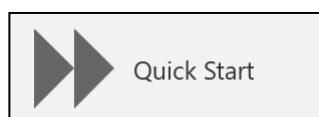
A layout is a table in a spreadsheet file (Excel format, .xlsx) which describes the samples to be measured. Layouts can be re-used to simplify recurring workflows. Depending on the type of experiment, different annotations in the layout are required. Refer to the table below for more information.

- For a **thermal unfolding** experiment, PlateID and WellID columns are required. Additional columns and annotations are optional.
- For a **chemical unfolding** experiment the following columns are required: PlateID, WellID, Sample ID, MeasurementTemperature, DenaturantName, DenStartConc, and DenEndConc. Additional columns and annotations are optional.

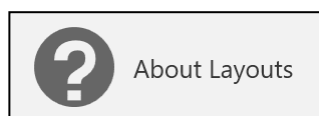
NOTE: Any additional annotations in optional columns will be visible in the PR.ThermControl or PR.ChemControl programs during data analysis. The pre-defined Sample ID column in the software will be used if a column with this name is specified in the layout.

Column name	Required information
PlateID	Number of the microtiter plate(s) (1-4) containing the samples to be measured. The format needs to conform to one of the following: “#”, “Plate#”, “MTP#”.
WellID	ID of the wells containing the samples to be measured, on the plate specified in the PlateID column. Specify the row letter or an individual well within the row to be measured (“A”, “A1”, “B12” etc.). NOTE: All wells in the specified rows will be measured. Measuring specific wells is not possible in the NT.RA setup.
Sample ID	Describing the protein in the sample. Further custom annotation columns can be specified.
Measurement Temperature	Defining the temperature at which the measurement is performed. Column and content are required.
DenaturantName	Describing the denaturant in the sample. Column is required, but annotations are optional.
DenStartConc	Short for Denaturant Start Concentration. Typically, the highest denaturant concentration in the assay, used in capillary 1. The unit used is molar [M].
DenEndConc	Short for Denaturant End Concentration. Typically, the lowest denaturant concentration in the assay, used in capillary 24. The unit used is molar [M].

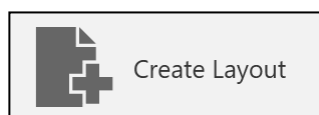
When starting PR.AutoThermControl or PR.AutoChemControl, the user is given different options for handling the measurement layout:



Quick Start allows to start a measurement without using a pre-defined layout.



About Layouts provides information on layouts and how to use them.



Create Layout opens a spreadsheet software with a pre-defined layout containing all required columns. Additional columns can be added. Information can be copied and pasted from other applications.



Import Layout allows to use a previously prepared layout (.xlsx format).

7.3. Modifying the Layout and Setting Plate Temperatures

No matter which Layout option is chosen, the subsequent screen allows to modify the selection of rows and plates to be measured (see Figure 8).

For NT.RA systems equipped with the Plate Temperature Control Option (see section 6.3), this screen also provides the option to define temperatures for the individual plate positions. After entering the desired plate temperature, click *Set* to implement. Click *Off* to disable temperature control for a specific plate. Use the lock button to switch between setting plates individually or all 4 plates at the same time.

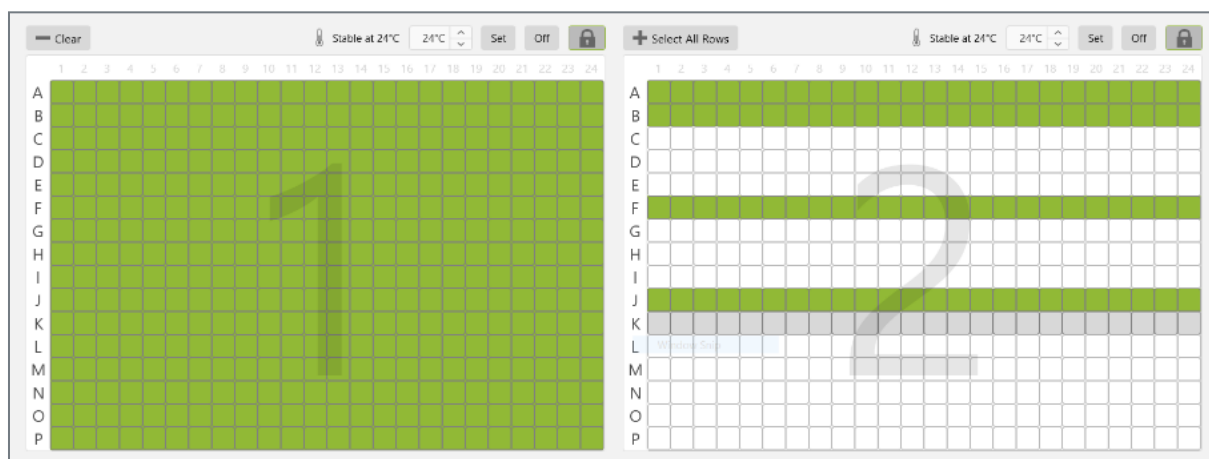


Figure 8: Part of the *Quick Start* screen. Select plates/rows to be measured, and define plate temperatures (temperature control only available for NT.RA systems equipped with Plate Temperature Control Option).

7.4. Experimental Parameters

After a layout is defined, the software asks the user to set the experimental parameters described below. Some differ depending on the software package used, while others are the same across packages. Fields with green background are required.

Please be aware that the plate position temperature is not considered an experimental parameter and therefore can be defined on the preceding screen (see section 7.3; only available on NT.RA systems equipped with Plate Temperature Control Option).

7.4.1. For both PR.AutoThermControl and PR.AutoChemControl

In this screen (see 7.4.2 and 7.4.3), define global settings for all samples in the entire measurement. Alternatively, individual sample/plate rows can be measured using specific settings. To do this, click the *Manual Settings* button in the bottom right-hand corner.

NOTE: If you used a pre-defined layout file, some of the experimental parameters will be automatically defined by the values in the layout file.

Set the excitation power for the measurement. Use *Auto Detect* for automatic determination of suitable excitation power. Note that the system re-determines the excitation power for every capillary chip. If the initial fluorescence of the samples varies, using the *Auto Detect* function may lead to different excitation power settings for different capillary chips measured. Alternatively, define the excitation power between 1 % and 100 % manually.

Click *Browse* to define the name and the location of the output file. Create a new output file or add measurements to a previously created file. Each measured capillary chip is displayed as a separate experiment in the *Project Overview* panel of PR.ThermControl and PR.ChemControl software (see 7.7). An output file can contain up to 64 chips.

If desired, the start of the measurement can be delayed. Enter the desired number of minutes. Clicking *Start Measurement* then starts a countdown of the delay time, after which the measurement is started automatically.

7.4.2. For PR.AutoThermControl

For a **thermal unfolding** experiment, define the start and end temperature for the melting scan, and set the temperature slope to the desired heating rate. The software will indicate the estimated duration of the measurement.

Activate the *Use Refolding Ramp* checkbox to include a refolding experiment with temperature slopes identical (but reversed) to the unfolding experiment.

7.4.3. For PR.AutoChemControl

For a chemical unfolding experiment, the name of the target protein and denaturant are optional. Start and end concentrations of the denaturant (highest and lowest concentrations) are required.

7.5. Starting a Measurement

Press *Start Measurement*. An information window will pop up, giving final instructions on NT.RA setup. Complete all points as requested to ensure proper operation of the NT.RA.

Click *Start* to start the measurement.

7.6. During a Measurement

While a measurement is running, the *System Monitor* provides information about the progress of the measurement.

It is possible to follow data acquisition in real time during the measurement using a temporary data file. This temporary data file is not saved, therefore annotations or other changes made in it will be lost upon closing.

To open a one-time temporary data file, click *View Data*. To automatically open a temporary data file for each measured chip, check the box of *View Automatically*. Note that these automatically opened files will also close automatically before the next one is opened.

7.7. Data Analysis

After the measurement is finished, load data files in the PR.ThermControl or PR.ChemControl software. The PR.ThermControl software is dedicated to analyzing thermal unfolding (and optionally refolding) experiments. The PR.ChemControl software is dedicated to analyzing chemical unfolding experiments. Each measured capillary chip is displayed as a separate experiment in the *Project Overview* panel of PR.ThermControl and PR.ChemControl software. For more details on PR.ThermControl or PR.ChemControl software please refer to the respective user manuals.

7.8. PR.AutoThermControl-CFR for 21 CFR part 11/Annex 11 compliance

PR.AutoThermControl-CFR software is compatible with PR.ThermControl-CFR, but not with PR.ThermControl software.

The PR.AutoThermControl-CFR software offers the same functionalities as PR.AutoThermControl and includes additional features that enable compliance with 21 CFR part 11 (FDA) and Annex 11 (EU) regulations. In accordance with these regulations, user permissions can be defined and

restricted for PR.AutoThermControl-CFR software using the PR.ThermControl-CFR Setup tool. Individual users may therefore not have the permission to execute all functions detailed in this user manual. For more information on managing user permission levels, please see the PR.ThermControl-CFR Setup software manual.

Analysis and reporting of acquired data is performed with PR.ThermControl-CFR. Furthermore, all activities carried out with the PR.AutoThermControl-CFR software are tracked in audit trails accessible via the PR.ThermControl-CFR software. Please refer to the PR.ThermControl-CFR manual for details.

The following subsections explain specifics of the PR.AutoThermControl-CFR software.

7.8.1. Installation and Installation Qualification

All software packages are installed and verified (IQ) on the control computers by NanoTemper Technologies before shipment to the customer, if not otherwise arranged. Documentation of this installation qualification procedure is provided upon request.

7.8.2. Locking the screen during a measurement

Once a measurement has been started and the robotic arm starts moving, the user can walk away. Press the Windows key + L to lock the screen of the control PC to prevent access by unauthorized users. During screen lock, the measurement will continue. If another user logs in during this time, they will neither be able to change the measurement nor use the NT.RA for other purposes, but they will be able to cancel the measurement via the PR.ThermControl-CFR user interface.

7.8.3. Measurement settings templates

Measurement settings can be saved and re-loaded for use in future measurements by choosing *Save Settings* or *Load Settings* in the *Define Measurement Settings* window. They contain a single set of experimental parameters (see 7.4), which will be used for all capillary chips of a measurement series, as well as settings for the plate position temperature (requires plate-temperature control, see 6.3).

They will be stored in .patct format in the secured data folder and can be exported to .pdf via the *Export Settings* dialogue in the same window.

7.8.4. Enclosure opening

When the enclosure is opened during an ongoing measurement series, the robotic arm pauses its movement while any ongoing measurement in the Prometheus NT.Plex continues (see 5.2.2.) The movement only resumes after closing the door and after user interaction in the PR.AutoThermControl-CFR interface. Only the user, who started the measurement series, will be presented with a pop-up window to continue the measurement and to provide a reason for the

enclosure opening. This functionality ensures integrity of the experimental setup and the resulting data and also tracks any unauthorized access to the system while the PR.AutoThermControl-CFR user has walked away.

Enclosure openings will be logged in the audit trail and the user will see a notification about it in all subsequent experiments of the ongoing measurement series in PR.ThermControl-CFR software. Please see the PR.ThermControl-CFR manual for details.

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